

**CLAIMS:**

1. A method for determining one or more kinetic parameters of binding between a first binding member and a second binding member comprising:
  - (a) adsorbing the first binding member to a surface at a plurality of microspots;
  - (b) presenting the second binding member to the first binding member at each of the microspots, there being a plurality of combinations of first binding member surface density and second binding member concentration among the plurality of microspots;
  - (c) simultaneously obtaining data indicative of a binding reaction between the first and second binding members at each of the plurality of microspots by a biosensor detection method; and
  - (d) processing the data so as to obtain one or more kinetic parameters of binding between the first and second binding members.
2. The method according to Claim 1 wherein the biosensor detection method is selected from surface plasmon resonance (SPR), critical angle refractometry, total internal fluorescence (TIRF), total internal reflection phosphorescence, total internal reflection light scattering, evanescent wave ellipsometry, and Brewster angle reflectometry.
3. The method according to Claim 1 or 2 wherein the detection method is SPR and the data indicative of a binding reaction between the first and second binding members at each of the plurality of microspots is an SPR parameter selected from the SPR resonance angle, resonance wavelength, reflectance changes, and phase changes.
4. The method according to Claim 1, 2 or 3, wherein the one or more kinetic parameters are selected from an association constant  $K_a$  a dissociation constant  $K_d$  and an affinity constant.

- 30 -

5. The method according to any one of Claims 1 to 4 wherein adsorbing the first binding member to a microspot comprises:

- (a) activating the surface in the microspot;
- (b) adsorbing the first binding member to the microspot; and
- 5 (c) deactivating the microspot.

6. The method according to Claim 5, wherein the step of activating the microspot involves presenting a chemical activating substance to the microspot.

7. The method according to Claim 6 wherein the step of presenting a chemical activating substance comprises

10 (a) forming a first channel around a region containing the microspot;

- (b) introducing a solution containing the activating substance into the channel; and
- (c) removing excess activating solution from the channel;

15 8. The method according to Claim 5 or 7 wherein the step of adsorbing a molecular species to the microspot involves:

- (a) forming a channel around a region containing the microspot ;
- (b) introducing a solution containing the molecular species into the channel; and
- 20 (c) removing excess solution from the channel;

9. The method according to Claim 5, wherein the step of activating the microspot involves producing an electric field over the microspot.

10. The method according to Claim 5, 6, or 7 wherein the molecular species is a first binding member and the method further comprises:

25 (a) deactivating portions of the surface not included in a microspot;

- (b) forming one or more second channels perpendicular to one or more of the first channels; and
- (c) for each second channel, introducing into the second channel

30 a second binding member.

- 31 -

11. The method according to any one of the previous claims further comprising obtaining reference data from a region of the surface not included in a microspot.

12. A method for localizing a molecular species at each of two or more microspots on a surface, comprising, for each of one or more localization regions:

- 5 (a) activating the surface in the localization region;
- (b) for each of one or more microspots in the localization region, adsorbing a molecular species to the microspot; and
- (c) optionally deactivating the localization region.

13. The method according to Claim 12, wherein the step of activating the 10 surface involves:

- (a) forming a first channel around the localization region;
- (b) introducing a solution containing an activating substance into the channel; and
- (c) removing excess activating solution from the channel;

15 14. The method according to Claim 12, wherein the step of activating the microspot involves producing an electric field over the microspot.

15. The method according to Claim 12 wherein the step of adsorbing a molecular species to the microspot involves:

- (a) forming a channel perpendicular the localization region;
- 20 (b) introducing a solution containing the molecular species into the channel; and
- (c) removing excess solution from the channel;

16. The method according to Claim 15 wherein the molecular species is a first binding member and the method further comprises:

- 25 (a) deactivating portions of the surface not included in a localization region;
- (b) forming one or more second channels perpendicular to one or more of the first channels; and
- (c) for each second channel, introducing into the second channel 30 a second binding member.

17. A probe array produced by the method of any one of Claims 12 to 16.
18. A system for simultaneously monitoring a plurality of binding reactions between one or more probe species and one or more target species comprising
  - (a) A surface;
  - 5 (b) An applicator capable of applying probe species to microspots on the surface so as to allow the probe species to be adsorbed to the microspot, the applicator being further capable of presenting a target to each probe species adsorbed to the surface;
  - 10 (c) A photosurface receiving light reflected from the surface and generating signals indicative of the binding of the targets to the probes; and
  - (d) A processor configured to receive the signals generated by the photosurface and to analyze the signals so as to produce a kinetic analysis of the binding.
- 15 19. The system according to Claim 18 wherein the signals are generated in a detection method selected from surface plasmon resonance (SPR), critical angle refractometry, total internal fluorescence (TIRF), total internal reflection phosphorescence, total internal reflection light scattering, evanescent wave ellipsometry, and Brewster angle reflectometry.
- 20 20. The system according to Claim 19 wherein the detection method is SPR and the data indicative of a binding reaction between the first and second binding members at each of the plurality of microspots is an SPR parameter selected from the SPR resonance angle, resonance wavelength, reflectance changes, and phase changes.
- 25 21. The system according to Claim 18, 19, or 20, wherein the kinetic analysis comprises obtaining one or more kinetic parameters selected from an association constant  $K_a$ , a dissociation constant  $K_d$  and an affinity constant.
22. The system according to any one of Claims 18 to 22 wherein an activatable region is activated by producing an electric field over the region.

23. The system according to any one of Claims 18 to 22 wherein an activatable region is activated by applying a chemical activator to the activatable regions.

24. The system according to any one of Claims 18 to 22 adapted to allow a probe species to be adsorbed to the surface in at least two different concentrations  
5 and is further adapted to allow a target species to be presented to the probe in at least two different concentrations.

25. The method according to Claim 24 wherein the processor is further configured to analyze the signals so as to determine a parameter indicative of the kinetics of binding of the probe to a target.

10 26. The system according to Claim 25 wherein the parameter indicative of the kinetics of binding of the probe to a target is an association constant or dissociation constant or affinity constant or any other used constants or value.

27. The system according to any one of Claims 17 to 25 having a flow cell comprising a plurality of microchannels, the flow cell being positionable on the  
15 surface so that a fluid flowing in a microchannel flows in contact with a region of the surface.

28. The system according to Claim 26 wherein the flow cell is positionable in a first position and in a second position, the microchannels when the flow cell is in the first position being perpendicular to the microchannels when the flow cell is in  
20 the second position.